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Isozyme Polymorphisms in Carob Cultivars

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Abstract. **The variability of isozymes in nine enzyme systems was studied in 25 carob (*Ceratonia siliqua* L.) cultivars using starch gel electrophoresis of leaf extracts. Five enzymes (phosphoglucomutase, phosphoglucoisomerase, aspartate aminotransferase, shikimic dehydrogenase, and aconitase) were polymorphic, making it possible for the 25 cultivars to be classified into eight phenotype categories.**

The carob is a leguminous tree native to the Mediterranean basin and southwestern Asia (Smith, 1976). The beans and kernels of this woody perennial are useful for a variety of purposes including food, fodder, and as a source of industrial products such as gums, sugar, and alcohol (Carlson, 1986). The major carob producing countries are Cyprus, Italy, Greece, Morocco, Portugal, and Spain, the latter being the world's largest producer and exporter of carob beans.

Morphological and physiological characters have been traditionally used for the identification of carob cultivars (Tous and Batlle, 1990). Some of them are strongly influenced by the environment, and their use may lead to unreliable or erroneous determinations. Futhermore, variation in fruit and flower characters, which is often important in the definition of a cultivar, can be discerned only in adult trees, precluding their use in the identification of young plants.

These problems can be avoided by using isozymes, because isozyme banding patterns are generally unaffected by the environment, and the phenotype of each cultivar can be rapidly determined with a small sample of plant tissue (leaf, root, pollen, etc.). For these reasons, gel electrophoresis of isozmyes has been developed as a method for cultivar identification in many tree crops. The objectives of this research were to describe the isozyme patterns for 25 carob cultivars, gain insight into the level of isozyme variation present in this species, and evaluate the efficiency of isozymes for cultivar identification.

The cultivars used in this study (Table 1) are part of the carob germplasm collection at the Centre de Mas Bové. Twenty-two of them were of Spanish origin, two were from Portugal, and one from Cyprus.

Actively growing leaves from tender shoots were collected and kept at 0 to 4C until enzyme extraction (within 2 h). Leaf samples of ≈1 cm^2 were crushed in 100 µl of the following extraction buffer (modified from Arulsekar and Parfitt, 1986): 0.05 M tris, 0.007 M citric acid, 0.1% ascorbic acid, 0.1% cysteine HCL, 1% polyethylene glycol, 1 mM 2-mercaptoethanol, and 8% polyvinylpyrrolidone (PVP-40). A piece (8 × 3 mm) of Whatman no. 3 filter paper was then saturated with the extract and inserted into a horizontal starch gel (11.5% Connaught hydrolyzed starch).

Histidine pH 7.0 (H7.0), histidine-citrate pH 5.7 (HC5.7), tris-citrate pH 7.8 (TC7.8), and tris-citrate pH 7.0 (TC7.0) gel buffers were used. The first three correspond, respectively, to buffers E, B, and F described by Shields et al. (1983). Gel buffer for TC7.0 consisted of 4 mM citric acid adjusted to pH 7.0 with tris, and the electrode buffer was 0.3 M boric acid adjusted to pH 8.2 with NaOH. HC5.7 and H7.0 gels were run for 2.30 to 3 h at 200 V, and TC7.8 and TC7.0 gels for 3 to 4 h at 300 V.

Staining solutions for phosphoglucoisomerase (PGI; EC 5.3.1.9), phosphoglucomutase (PGM; EC 2.7.5.1), 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), malate dehydrogenase (MDH; EC 1.1.1.37), isocitrate dehydrogenase (IDH, EC 1.1.1.42), shikimic dehydrogenase (SDH, EC 1.1.1.25), aspartate aminotransferase (AAT; EC 2.6.1.1), and leucine aminopeptidase (LAP; 3.4.11.1) were as described by Vallejos (1983). For aconitase (ACO; EC 4.2.1.3), gels were stained in 75 ml 0.1 M tris pH 8.0, 60 mg cis-aconitic acid, 15 mg thiazole blue (MTT), 6 mg nicotinamide adenine dinucleotide phosphate (NADP), 4 mg phenazine methosulfate (PMS), 4 ml 10% $MgCl_2$ and 50 µ IDH. HC5.7 gels were used for ACO, 6PGD, MDH, and SDH, H7.0 for

Table 1. Isozyme phenotypes for 25 carob cultivars.

Cultivar	Region of activity					Sex[z]	Origin[y]	I[x]
	PGM-2	PGI-2	SDH-1	ACO-1	AAT-1			
Banya de Cabra	1	A	12	2	1	F	S(B)	a
Barenys	1	C	2	2	1	M	S(T)	*
Cacha	1	A	2	2	12	F	S(V)	b
Costella de Ruc	1	A	2	2	12	F	S(T)	b
Duraió	1	A	2	2	1	F	S(M)	c
Forastera	1	A	12	2	1	H	S(M)	a
Galhosa	1	A	2	12	12	F	P	*
La Plana	1	A	2	2	1	M	S(T)	c
Mascle Groc	1	A	12	2	2	M	S(T)	*
Matalafera	1	A	2	2	1	F	S(V)	c
Misto 1	1	A	12	2	1	H	S(T)	a
Misto St. Barbara	1	A	2	2	12	H	S(T)	b
Molí	1	A	12	2	1	H	S(T)	a
Mulata	1	A	2	2	12	F	P	b
Negra	1	A	2	2	12	F	S(T)	b
Ramillete	1	A	2	2	1	H	S(MU)	c
Ralladora	1	A	2	2	12	F	S(C)	b
Rochal	1	A	12	2	12	F	S(A)	d
Roja	1	A	12	2	12	F	S(C)	d
Rojal	1	A	2	2	1	F	S(T)	c
Santa Cirga	1	A	12	2	1	H	S(M)	a
Tendral	1	A	2	2	1	F	S(T)	c
Tylliria	12	B	2	2	12	F	CY	*
Valencià	1	A	2	2	1	F	S(T)	c
Vera	1	A	2	2	12	F	S(A)	b

[z]Sex: H, hermaphrodite; M, male; F, female.
[y]Origin: S = Spain (area of production in parentheses: A = Alicante, B = Barcelona, C = Castellón, M = Mallorca, MU = Murcia, T = Tarragona, V = Valencia); CY = Cyprus; P = Portugal.
[x]I = identification. Isozyme notation: * Isozyme phenotype unique; letters a–d: cultivars with the same letter have identical isozyme phenotypes.

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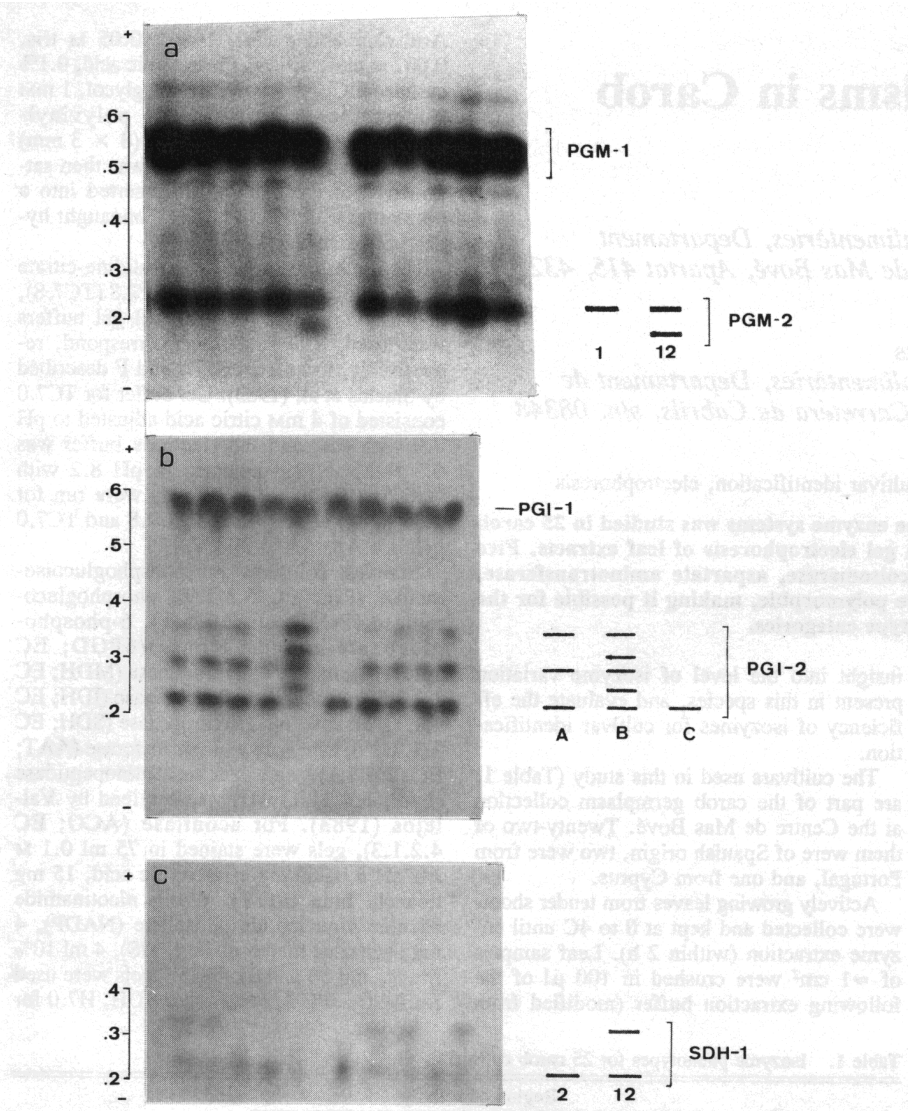

Fig. 1. Photographs and interpretative drawings for PGM (a), PGI (b), and SDH (c) isozymes. Rf values are given in the vertical scale. Cultivars are (left to right): 'Roja', 'Rochal', 'Negra', 'Valencià', 'Tylliria', 'Barenys', 'Mascle groc', 'Misto 1', 'Cacha', and 'Molí'.

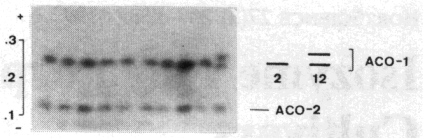

Fig. 2. Photograph and interpretative drawing for ACO isozymes. Rf values are given in the vertical scale. Cultivars are (left to right): 'Barenys', 'Mascle groc', 'Misto 1', 'Cacha', 'Molí', 'Duraió', 'Matalafera', 'La Plana', 'Mulata', and 'Galhosa'.

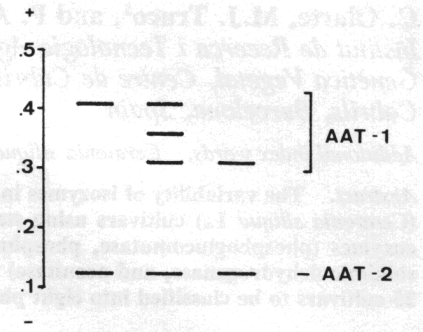

Fig. 3. Schematic zymogram of AAT isozymes. Rf values are given in the vertical scale.

IDH and PGM, TC7.8 for AAT and LAP, and TC7.0 for PGI.

All of the cultivars had the same banding patterns for the enzymes LAP, 6PGD, MDH, and IDH. Variability was detected in the remaining five enzyme systems.

Phosphoglucomutase. Two zones of activity (PGM-1 and PGM-2) were observed in gels stained for this enzyme (Fig. 1a). PGM-1 was apparently monomorphic, and PGM-2 was variable with two electromorphs (1 and 2), each cultivar having either band 1 or both bands.

Phosphoglucoisomerase. There were two regions of PGI activity: PGI-1 and PGI-2 (Fig. lb). Variability was found in PGI-2, where it was possible to distinguish three phenotypes, A, B and C, with three, five, and one bands, respectively.

Shikimic dehydrogenase. A single region of activity (Fig. 1c) with two phenotypes, one with one band and the other with two bands, occurred in this enzyme system.

Aconitase. Two ACO active zones designated ACO-1 and ACO-2 were present (Pig. 2). All cultivars had one band in each region, except 'Galhosa', which had an additional band in ACO-1.

Aspartate aminotransferase. Two weakly stained regions of activity were found in the zymogram of AAT (Fig. 3). Variability was detected in only one of them, AAT-1, with three phenotypes observed: two with a single band (1 and 2) and one with three bands (1, 2, and a band of intermediate mobility).

The level of isozyme variation among the 25 cultivars analyzed was low. Of the five variable regions of activity observed, three (ACO-1, PGM-2, and SDH-1) had only two phenotypes, and the less frequent phenotype of ACO-1 and PGM-2 was found in only one cultivar. Therefore, the usefulness of isozymes for cultivar identification was low; only four cultivars of the 25 studied possessed unique phenotypes (Table 1). The remainder could be grouped into four classes, two with seven cultivars each, one with five, and one with two cultivars.

An interesting observation emerges when the phenotypes of the 22 Spanish cultivars are compared with those of three cultivars from other regions; the former were only variable for PGI-2 and AAT-1, whereas the latter were polymorphic for all variable regions of activity detected, having unique phenotypes for PGM-2 and PGI-2 (cv. Tylliria) and for ACO-1 (cv. Galhosa). These results suggest that Spanish cultivars may be a genetically impoverished sample of variation from the species as a whole. The study of a broader sample of genotypes, including a balanced representation of cultivars from countries where carob is grown, could provide a better estimation of the level and pattern of geographic distribution of isozyme variation of this species. In addition, such research might uncover new polymorphic isozmyes potentially useful as markers for the genetics and breeding of this crop.

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